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OCCURRENCE OF FRUIT-ROT OF CHILLI IN SINDH AND THEIR BIOMANAGEMENT UNDER LABORATORY CONDITIONS

FAISAL HUSSAIN[1*] MUHAMMAD ABID[1], IRAM US SALAM[1], MUHAMMADI[2], FARZANA[1], MUHAMMAD AKBAR[3] AND ALAMDAR HUSSAIN[4]

[1]Dr. A.G. Laboratory of Aerobiology and Plant Pathology, Department of Botany, Federal Urdu University of Arts, Science and Technology, Gulshan-e-Iqbal Campus, Karachi 75300 Pakistan
[2]Department of Physical Health, Islamabad Business School, DHA phase-II, Gate III, GT road, Islamabad, Pakistan
[3]Department of Environment Sciences, Karakorum International University, Gilgit-Pakistan
[4]Department of Biological Sciences, Karakorum International University, Gilgit-Pakistan
[*]Correspondence author's email: faisal.botanist2011@gmail.com

Abstract

The fungal pathogens were isolated from the affected samples of Chilli plants collected from different areas of Sindh. The affected fruits/pods of chilli were collected from the centrally located large godowns and small storing units for the identification and isolation of fungi. Fruits/pods were significantly infected by *Aspergillus candidus, A. flavus, A. fumigatus, A. niger* and *A. terreus*. During the study, it was observed that *Aspergillus flavus, A. niger* and *A. terreus* were extensively and intensively infecting the fruit of Chilli crop. Four antagonistic fungi, *Gliocladium virens, Paecilomyces lilacinus, Penicillium commune* and *Trichoderma harzianum* were screened against the above mentioned plant pathogenic fungi *In vitro* which suppressed the growth of pathogenic fungi. In addition, it disclosed that *T. harzianum* and *P. lilacinus* were found antagonistic against *Aspergillus candidus, A. flavus, A. terreus* and *A. niger* as it resulted a strong suppressive effect on the growth and mycelial development.

Keywords: Chilli, *Aspergillus*, Lower regions, Fruit contamination, Antagonism, Bio-control.

Introduction

There are number of *Aspergillus* species are recorded in Chilli crop growing areas of Pakistan including *Aspergillus flavus, A. niger, A. fumigatus* etc. The genus *Aspergillus* is considered more than 180 amorphic species in all over the world (Pitt *et al.*, 2000). It is a filamentous fungus. This fungus survived on decaying vegetation, dead leaves, stored fruits and seeds and also creates problem in stored grain, after harvesting and during processing dryness of Chilli crop (Hussain, 2013). The spores of this fungus are wide spread and available in the air everywhere. *A. niger* is also responsible for mycotoxicity such as ochratoxin A and aflatoxins etc and food spoilage (Kozakiewicz, 1989; Hussain *et al.*, 2013a; 2013b). This fungus caused significant losses in Chilli crop throughout the world wide. The long stalk conidiophores with vesicle were observed like other *Aspergillus* species. Vesicle produces the globose conidia, which are the source of dispersal from one host to another. This pathogen is considered for the contamination of Chilli fruit and it is also authentic source of serious toxins such as aflatoxin. *Aspergillus* is also rich source of mycotoxins particularly aflatoxin is produced by this genus. Many strains of *Aspergillus* may generate significant effect of aflatoxin, a carcinogenic poisonous compound (Klich, 2007). This fungus usually grows on fruit development and drying of fruit when fruits have some moisture (Hussain & Abid, 2011; Hussain, 2013; 2013a; 2013b). Samples of Chillies from different sources yielded several colonies contain numerous species of *Aspergillus* (Christensen *et al.*, 1967, Hussain *et al.*, 2013a; 2013b). Aflatoxins are chemically classified in secondary metabolite which is mostly produced by *Aspergillus bombycis, A. flavus, A. nomius, A. parasiticus* and *A. tamarii* (Kurtzman *et al.*, 1987; Goto *et al.*, 1997; Peterson *et al.*, 2001).

Some *Trichoderma* species are considered affective as a biocontrol agent against different pathogenic fungi (Chet *et al.*, 1998; Howell, 1998; Siddiqui *et al.*, 2001). In particular, *Trichoderma harzianum* has been demonstrated a very suppressive biocontrol agent (Zeilinger *et al.*, 1999; Siddiqui & Shaukat, 2004). Antagonistic interactions are recognized as one of the most mechanisms for biocontrol of fungal pathogens (Khara & Hadwan, 1990; Hussain *et al.*, 2013a). Biocontrol agent *T. harzianum* is commercially produced to prevent the growth of different soil-borne pathogens (Shalini *et al.*, 2006). *Paecilomyces* is well known biological control agent against root rot and root-knot infecting pathogens (Hasan & Jain, 1992). The result of soil amendment with medicinal plants is effective with combination of biocontrol agents such as *P. lilacinusa* and *Pseudomonas aeruginosa* in decreasing of infection by root rot and root-knot infecting pathogens of mungbean (Mansoor *et al.*, 2007). The use of biocontrol agents such as *Paecilomyces* (Jatala, 1985) and rhizobia also provide significant manage of root rot and root-knot infecting pathogens (Ehteshamul-Haque & Ghaffar, 1993; Ehteshamul-Haque *et al.*, 1996).

The main objectives of the present study were 1) to survey the fungi infecting chilli fruits in godowns and large as well as small storing units; and to find out the dominant fungi of storage-rot chilli fruit and 2) to investigate the biocontrol potential of some fungi against storage-rot fungi.

Methods and Materials

Collection of infected fruits: The fruits/pods of Chilli were collected from the large and small uniting areas and different godowns of lower regions of Sindh province in Pakistan including Hyderabad, Tando Allahyar, Mirpurkhas, Umerkot, Kunri, Samaro, Kot Ghulam Muhammad and Digri were taken from August to

December 2014. The infected fruit samples were surfaces were sterilized by 1% Calcium hypochlorite for 1 min and transferred on PDA medium containing anti-bacterial (Penicillin and Streptomycin). The Petri dishes were kept in incubator for 5 days at 28 ± 2°C.

Identification of fungi: The causal organisms isolated and identified using standard references (Ellis, 1971; 1976; Barnett and Hunter, 1972; Domsch *et al.*, 1980; Sutton, 1980; Nelson *et al.*, 1983; Singh *et al.*, 1991).

Screening of antagonistic fungi against *Aspergillus* species: *Gliocladium virens*, *Paecilomyces lilacinus*, *Penicillium commune* and *Trichoderma harzianum* were used as test fungi against *Aspergillus flavus, A. fumigatus, A. terreus, A. niger* and *A. candidus*. Both antagonist and pathogenic fungi were inoculated at the contrary ends of the Petri plates containing 20ml PDA media. Three Petri dishes were kept for each treatment and same numbers of Petri dishes were kept as control with pathogen alone. The Petri dishes were incubated for six days at 30°C after inoculation of antagonistic and pathogenic fungi. The colony diameters of both antagonistic and pathogenic fungi were recorded every day up to 6 days. Colony diameter of control was also calculated. The measured colony diameter (mm) in which there is interaction of the different pathogens with *G. virens*, *P. lilacinus, P. commune* and *T. harzianum* were observed. The data of inhibition percentage of radial growth were observed and the zone of inhibition (ZI) is given below (Royse & Ries, 1977; Whips, 1987; Reddy & Hynes, 1993).

$$\text{Inhibition (\%)} = \frac{Y - Z}{Y} \times 100$$

where Y = Mycelia growth of pathogen alone (control),

Z = Mycelia growth of pathogen with antagonist

Results and Discussion

Five fungal species of *Aspergillus* were isolated from infected samples of chilli fruits. The result of the present study showed that *Aspergillus flavus, A. niger* and *A. fumigates* were all over dominant, respectively as compared to other species. Present studies showed that *Aspergillus flavus, A. niger* and *A. fumigatus* on fruit (that cause spoilage of fruit particularly at post-harvest and during storage of fruit) were dominant high occurrence % in samples collected from Kunri (77%), Umerkot (70%) and Samaro (69%) respectively while minimum (18%) from Digri region (Fig. 1).

The results observed from Fig. 1 are representing the severity of fungal occurrence percentage on chili fruits at various localities of lower Sindh. The occurrence percentage of *A. flavus* was also observed much higher than other fungal species at Kunri locality and Umerkot locality was observed second most infested locality. The occurrence percentage of *A. candidus* was observed lowest among all fungi in all localities. However, the Kunri locality gave more significantly higher incidences of infestation. The localities of Kot Ghulam Muhammad and Digri have shown lowest incidences. The results of ANOVA for occurrence percentage of Chilli fruits were observed in various localities. All five fungal species including *Aspergillus candidus* ($F=22.13$, $p<0.001$), *A. flavus* ($F=15.82$, $p<0.001$), *A. fumigates* ($F=25.96$, $p<0.001$), *A. niger* ($F=69.66$, $p<0.001$) and *A. terreus* ($F=25.96$, $p<0.001$) showed highly significant differences among localities.

It was observed that *Aspergillus flavus, A. niger* and *A. fumigates* were extensively and intensively infecting the chilli fruit. The infections were increased rapidly due to many factors such as, infection through godowns where chillies stored, presence of moisture and temperature, small storage dumps, and poor practices of drying chillies openly near chilli fields. In addition to these increased levels of infection may be due to dispersal of fungal spores through winds, gales and dust storms as well as by mechanical vectors. Mushtaq & Hashmi (1997), Nahar *et al.* (2004) and Ahmad *et al.* (1997) also reported fruit and foliar fungi species such as; *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, A. terreus, Cercospora capsici* and *Colletotrichum capsici* from different localities of Pakistan with little bit similar results. It is interesting to note that Karachi, located in southern Sindh, studies on airborne mycobiota (Afzal *et al.*, 2004 and Rao *et al.*, 2009) have demonstrated that the aerospora is dominated by *Aspergillus niger, A. flavus, A. candidus, A. fumigatus* and *Alternaria solani*. Thus the atmospheric mycobiota tends to correspond with the chilli phylloplane and fruit-surface fungal dominance. According to Michalik (2007) the productivity of chilli depends not only on farmers and conditions of cultivations but also depends on weather conditions. Rainy and cloudy season heavy affected the chilli crop. Buczkowska & Bednarek (2005) have also reported and confirmed the above findings and explained that a high association between effective atmosphere temperature during cultivation and marketable productivity plays key role for farmers.

For screening of antagonism, some selected antagonistic fungi viz., *Gliocladium virens*, *Paecilomyces lilacinus*, *Penicillium commune* and *Trichoderma harzianum* were used as test fungi against *Aspergillus flavus, A. fumigatus, A. terreus, A. niger* and *A. candidus.* After six day inoculation, the zone of inhibition (ZI) was observed in mm. The result of ZI is given in Table 1.

During this test, *G. virens* proved to be useful antagonist against the growth of *Aspergillus terreus* by 3.7 mm. However, in the test of *P. lilacinus*, it was observed to be effective against the growth of *A. niger* by 2.6 mm (Table 1).

The antagonistic effect of *P. commune* on different fungi was observed relatively no effective in inhibiting the growth of *Apsergillus candidius, A. flavus, A. fumigates, A. niger* and *A. terreus*. When *T. harzianum* was tested against different species of *Aspergillus* species, it was noted as more effective and suppressive agent than other antagonists fungi. *T. harzianum* reduced and inhibited the growth of *A. flavus* and *A. terreus* by 3.9 mm and 2.7 mm, respectively. The results of ANOVA for antagonistic effect on different fungi were observed. Five fungal species including *Aspergillus candidus, A. flavus, A. fumigatus, A. niger* and *A. terreus* have shown significant differences ($F=4.22$, $p<0.005$) and inhibited by antagonistic fungi including *G. virens*, *P. lilacinus, P. commune* and *T. harzianum* ($F=7.60$, $p<0.001$). All five species proved as interacted with different fungi pathogens.

Table 1. Antagonistic fungi and their zone of inhibition (mm) on pathogenic fungi with Mean and S.E.

Pathogens	Zone of Inhibition (mm)			
	Gliocladium virens	***Paecilomyces lilacinus***	***Penicillium commune***	***Trichoderma harzianum***
Aspergillus candidus	A	B	A	A
A. flavus	B	B	A	3.9 ± 0.26
A. fumigates	B	B	A	B
A. niger	A	2.6 ± 0.15	B	B
A. terreus	3.7 ± 0.35	A	B	2.7 ± 0.17

A= Over growth, **B=** Growth stop

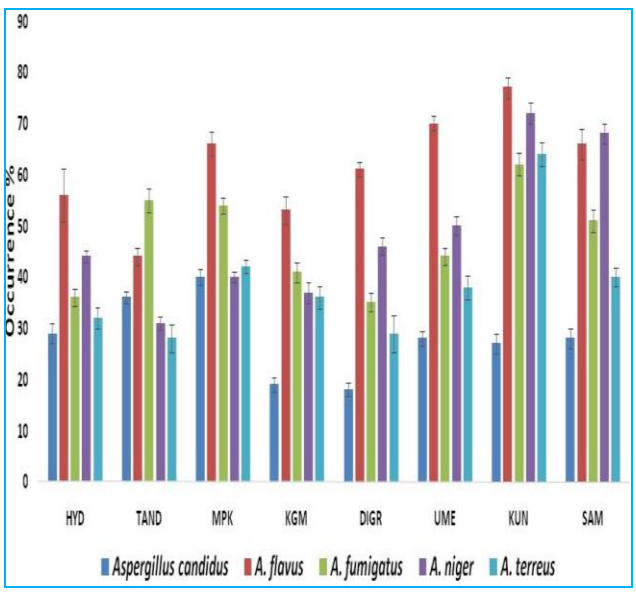

Fig. 1. Mean and Standard error of different fungi isolated from Chilli fruit at various localities (HYD= Hyderabad, TAND= Tando Allahyar, MPK= Mirpurkhas, KGM= Kot Ghulam Muhammad, DIG= Digri, UME= Umerkot, KUN= Kunri and SAM= Samaro) of lower Sindh- Pakistan.

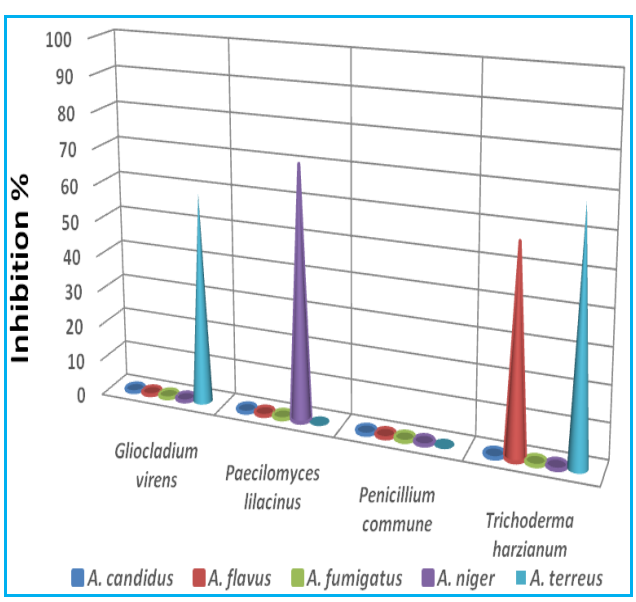

Fig. 2. Inhibition % of different antagonistic fungi against *Aspergillus* species.

Almost all antagonistic isolates inhibited the growth of *Aspergillus candidus, A. flavus, A. fumigatus, A. niger* and *A. terreus* except *P. commune* as shown in (Fig. 2). Among these antagonistic isolates, *P. lilacinus, T. harzanium,* and *G. virens* resulted as effective antagonist inhibiting the growth of fungi by 70%, 69% and 56.66% respectively as compared to remaining tested antagonist isolates. In contrast treatments, *P. commune* were not found effective all *Aspergillus* species as compared to the other antagonists (Fig. 2).

Muthamilan & Jeyarajan (1996) reported that biocontrol of pathogens by the utilizing of antagonistic fungi is possible for future and can be successfully launched particularly within the structure of disease management. Dandurand and Knudsen (1993) reported that the species of *Trichoderma* have been focused as most potent biocontrol agents for fungal diseases; other biotic factors such as bacteria have generally been considered as affect to their control activities. *T. harzianum* is recognized for degrading enzymes of plants (Vinale *et al.*, 2006). *P. lilacinus* has been reported as a mycoparasite (Kachuvaava, 1960) and also to decrease the survival germination of *Aspergillus parasiticus* and *A. flavus* sclerotia (Will *et al.*, 1994). However, *P. lilacinus* is considered egg-parasite of root-knot nematodes (Jatala, 1986). It has been also reported to reduce the infection by root-infecting pathogens (Ehteshamul-Haque *et al.*, 1995). Mansoor *et al.* (2007) reported that *P. lilacinus* in association with medicinal weed *Launaea nudicaulis* shows effective activity for the control of root rot and root-knot nematodes of mungbean and aggressively suppressed the several fungi. In the light of present investigation, *T. harzianum* and *P. lilacinus* were resulted as effective antagonists inhibiting the growth of pathogen. These results confirms the findings of Will *et al.* (1994), Wicklow (1987), Wicklow *et al.* (1990), Ehteshamul-Haque *et al.* (1995), Mansoor *et al.* (2007) and Gomathi & Ambikapathy (2011).

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